AGTCCCAGACGGGCTTTTCCCAGAGAGCTAAAAGAGAGGGCCAGAGAATGTCGTCCCAG 5 CCAGCAGGGAACCAGACCTCCCCGGGGCCACAGAGGACTACTCCTATGGCAGCTGGTAC ACCAGCATACCACCCGGCCTGTACCACGCCTGCCTGGCCTCGCTGTCAATCCTTGTGCTG CTGCTCCTGGCCATGCTGGTGAGGCGCCGCCAGCTCTGGCCTGACTGTGCGTGGCAGG CCCGGCCTGCCAGCCCTGTGGATTTCTTGGCTGGGGACAGGCCCCGGGCAGTGCCTGCT 10 GCTGTTTTCATGGTCCTCCTGAGCTCCCTGTGTTTGCTGCTCCCCGACGAGGACGCATTG CCCTTCCTGACTCTCGCCTCAGCACCCAGCCAAGATGGGAAAACTGAGGCTCCAAGAGGG GCCTGGAAGATACTGGGACTGTTCTATTATGCTGCCCTCTACTACCCTCTGGCTGCCTGT GCCACGGCTGGCCACACAGCTGCACACCTGCTCGGCAGCACGCTGTCCTGGGCCCACCTT GGGGTCCAGGTCTGGCAGAGGGCAGAGTGTCCCCAGGTGCCCAAGATCTACAAGTACTAC 15 TCCCTGCTGGCCTCCCTGCTGCTGGGCCTCGGATTCCTGAGCCTTTGGTACCCT GTGCAGCTGGTGAGAAGCTTCAGCCGTAGGACAGGAGCAGGCTCCAAGGGGCTGCAGAGC AGCTACTCTGAGGAATATCTGAGGAACCTCCTTTGCAGGAAGAAGCTGGGAAGCAGCTAC CACACCTCCAAGCATGGCTTCCTGTCCTGGGCCCGCGTCTGCTTGAGACACTGCATCTAC ACTCCACAGCCAGGATTCCATCTCCCGCTGAAGCTGGTGCTTTCAGCTACACTGACAGGG ₫20 ACGGCCATTTACCAGGTGGCCCTGCTGCTGCTGGTGGCGTGGTACCCACTATCCAGAAG GTGAGGGCAGGGTCACCACGGATGTCTCCTACCTGCTGGCCGGCTTTGGAATCGTGCTC TCCGAGGACAAGCAGGAGGTGGTGGAGCTGGTGAAGCACCATCTGTGGGCTCTGGAAGTG TGCTACATCTCAGCCTTGGTCTTGTCCTGCTTACTCACCTTCCTGGTCCTGATGCGCTCA CTGGTGACACAGGACCAACCTTCGAGCTCTGCACCGAGGAGCTGCCCTGGACTTGAGT 25 CCCTTGCATCGGAGTCCCCATCCCTCCCGCCAAGCCATATTCTGTTGGATGAGCTTCAGT GCCTACCAGACAGCCTTTATCTGCCTTGGGCTCCTGGTGCAGCAGATCATCTTCCTG GGAACCACGGCCCTGGCCTTCCTGGTGCTCATGCCTGTGCTCCATGGCAGGAACCTCCTG CTCTTCCGTTCCCTGGAGTCCTCGTGGCCCTTCTGGCTGACTTTGGCCCTGGCTGTGATC CTGCAGAACATGGCAGCCCATTGGGTCTTCCTGGAGACTCATGATGGACACCCACAGCTG GGTGCCATGGTGGCCACCTGGCGAGTGCTCCTCTCTGCCCTCTACAACGCCATCCACCTT GGCCAGATGGACCTCAGCCTGCTGCCACCGAGAGCCGCCACTCTCGACCCCGGCTACTAC ACGTACCGAAACTTCTTGAAGATTGAAGTCAGCCAGTCGCATCCAGCCATGACAGCCTTC TGCTCCCTGCTGCAAGCGCAGAGCCTCCTACCCAGGACCATGGCAGCCCCCCAGGAC 35 AGCCTCAGACCAGGGGAGGAAGACGAAGGGATGCAGCTGCTACAGACAAAGGACTCCATG GCCAAGGGAGCTAGGCCCGGGGCCAGCCGGGGGCAGGGCTCGCTGGGGTCTGGCCTACACG CTGCTGCACAACCCAACCCTGCAGGTCTTCCGCAAGACGGCCCTGTTGGGTGCCAATGGT ${\tt GCCCAGCCC}{\tt TGA}{\tt GGGCAGGGAAGGTCAACCCACCTGCCCATCTGTGCTGAGGCATGTTCC}$ TGCCTACCATCCTCCCCCGGCTCTCCCCAGCATCACACCAGCCATGCAGCCA 40 GGCTCTGCTCCACCCACTTGGCTATGGGAGAGCCAGCAGGGGTTCTGGAGAAAAAACTG GTGGGTTAGGGCCTTGGTCCAGGAGCCAGTTGAGCCAGGGCAGCCACATCCAGGCGTCTC CCTACCCTGGCTCTGCCATCAGCCTTGAAGGGCCTCGATGAAGCCTTCTCTGGAACCACT CCAGCCCAGCTCCACCTCAGCCTTGGCCTTCACGCTGTGGAAGCAGCCAAGGCACTTCCT 45 CACCCCTCAGCGCCACGGACCTCTCTGGGGAGTGGCCGGAAAGCTCCCGGTCCTCTGGC CTGCAGGGCAGCCCAAGTCATGACTCAGACCAGGTCCCACACTGAGCTGCCCACACTCGA GAGCCAGATATTTTTGTAGTTTTTATGCCTTTGGCTATTATGAAAGAGGTTAGTGTGTTC

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MSSQPAGNQTSPGATEDYSYGSWYIDEPQGGEELQPEGEVPSCHTSIPPGLYHACLASLS ILVLLLLAMLVRRRQLWPDCVRGRPGLPSPVDFLAGDRPRAVPAAVFMVLLSSLCLLLPD EDALPFLTLASAPSQDGKTEAPRGAWKILGLFYYAALYYPLAACATAGHTAAHLLGSTLS 5 WAHLGVQVWQRAECPQVPKIYKYYSLLASLPLLLGLGFLSLWYPVQLVRSFSRRTGAGSK GLQSSYSEEYLRNLLCRKKLGSSYHTSKHGFLSWARVCLRHCIYTPQPGFHLPLKLVLSA TLTGTAIYQVALLLLVGVVPTIQKVRAGVTTDVSYLLAGFGIVLSEDKQEVVELVKHHLW ALEVCYISALVLSCLLTFLVLMRSLVTHRTNLRALHRGAALDLSPLHRSPHPSRQAIFCW MSFSAYQTAFICLGLLVQQIIFFLGTTALAFLVLMPVLHGRNLLLFRSLESSWPFWLTLA 10 LAVILQNMAAHWVFLETHDGHPQLTNRRVLYAATFLLFPLNVLVGAMVATWRVLLSALYN AIHLGQMDLSLLPPRAATLDPGYYTYRNFLKIEVSQSHPAMTAFCSLLLQAQSLLPRTMA APQDSLRPGEEDEGMQLLQTKDSMAKGARPGASRGRARWGLAYTLLHNPTLQVFRKTALL GANGAQP Important features of the protein: 15 Signal peptide: None Transmembrane domain: 20 54-69 102-119 148-166 207-222 25 301-320 364-380 431-451 474-489 560-535 30 Motif file: Motif name: N-glycosylation site. 8-12 35 Motif name: N-myristoylation site. 50-56 176-182 40 241-247 317-323 341-347 525-531 627-633 45 631-637 640-646 661-667 Motif name: Prokaryotic membrane lipoprotein lipid attachment site. 50 364-375 Motif name: ATP/GTP-binding site motif A (P-loop).

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132-140

FIGURE 3A

PRO

XXXXXXXXXXXXXX

(Length = 15 amino acids)

Comparison Protein

XXXXXYYYYYYY

(Length = 12 amino acids)

5

10

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 15 = 33.3%

FIGURE 3B

PRO

XXXXXXXXX

(Length = 10 amino acids)

Comparison Protein

XXXXXYYYYYYZZYZ

(Length = 15 amino acids)

5

10

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 10 = 50%

15

FIGURE 3C

PRO-DNA NNNNNNNNNNNNN (Length = 14

nucleotides)

5 Comparison DNA NNNNNLLLLLLLLLL (Length = 16

nucleotides)

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

6 divided by 14 = 42.9%

FIGURE 3D

PRO-DNA

ИИИИИИИИИИИ

(Length = 12 nucleotides)

Comparison DNA

NNNNLLLVV

(Length = 9 nucleotides)

5

10

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

4 divided by 12 = 33 3%

FIGURE 4A

```
* C-C increased from 12 to 15
      5
            * Z is average of EQ
            * B is average of ND
            * match with stop is M; stop-stop = 0; I (joker) match = 0
            * f
            #define M
                                      /* value of a match with a stop */
    10
           int
                     day[26][26] = {
                   A B C D E F G H I J K L M N O P Q R S T U V W X Y Z */
           /*
           /* A */
                      { 2, 0, -2, 0, 0, -4, 1, -1, -1, 0, -1, -2, -1, 0, M, 1, 0, -2, 1, 1, 0, 0, -6, 0, -3, 0},
           /* B */
                      { 0, 3,-4, 3, 2,-5, 0, 1,-2, 0, 0,-3,-2, 2, M,-1, 1, 0, 0, 0, 0, -2,-5, 0,-3, 1},
    15
           /* C */
                      {-2,-4,15,-5,-5,-4,-3,-3,-2, 0,-5,-6,-5,-4, M,-3,-5,-4, 0,-2, 0,-2,-8, 0, 0,-5},
           /* D */
                      { 0, 3,-5, 4, 3,-6, 1, 1,-2, 0, 0,-4,-3, 2,_M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 2},
           /* E */
                       { 0, 2, -5, 3, 4, -5, 0, 1, -2, 0, 0, -3, -2, 1, M, -1, 2, -1, 0, 0, 0, -2, -7, 0, -4, 3},
           /* F */
                      {-4,-5,-4,-6,-5, 9,-5,-2, 1, 0,-5, 2, 0,-4, M,-5,-5,-4,-3,-3, 0,-1, 0, 0, 7,-5},
           /* G */
                      { 1, 0, -3, 1, 0, -5, 5, -2, -3, 0, -2, -4, -3, 0, M, -1, -1, -3, 1, 0, 0, -1, -7, 0, -5, 0},
   20
           /* H */
                      {-1, 1,-3, 1, 1,-2,-2, 6,-2, 0, 0,-2,-2, 2, M, 0, 3, 2,-1,-1, 0,-2,-3, 0, 0, 2},
           /* ] */
                      {-1,-2,-2,-2,-1,-3,-2, 5, 0,-2, 2, 2,-2, M,-2,-2,-1, 0, 0, 4,-5, 0,-1,-2},
           /* ] */
                      /* K */
                      {-1, 0,-5, 0, 0,-5,-2, 0,-2, 0, 5,-3, 0, 1, M,-1, 1, 3, 0, 0, 0,-2,-3, 0,-4, 0},
           /* L */
                      {-2,-3,-6,-4,-3, 2,-4,-2, 2, 0,-3, 6, 4,-3,_M,-3,-2,-3,-1, 0, 2,-2, 0,-1,-2},
/* M */
                      {-1,-2,-5,-3,-2, 0,-3,-2, 2, 0, 0, 4, 6,-2,_M,-2,-1, 0,-2,-1, 0, 2,-4, 0,-2,-1},
           /* N */
                      { 0, 2,-4, 2, 1,-4, 0, 2,-2, 0, 1,-3,-2, 2, M,-1, 1, 0, 1, 0, 0,-2,-4, 0,-2, 1}.
ŀå
           /* O */
                                                                ű
           0,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M},
                      \{1,-1,-3,-1,-1,-5,-1,0,-2,0,-1,-3,-2,-1,M,6,0,0,1,0,0,-1,-6,0,-5,0\},
           /* P */
   30
           /* Q */
                      { 0, 1,-5, 2, 2,-5,-1, 3,-2, 0, 1,-2,-1, 1, M, 0, 4, 1,-1,-1, 0,-2,-5, 0,-4, 3},
FL
           /* R */
                      {-2, 0, -4, -1, -1, -4, -3, 2, -2, 0, 3, -3, 0, 0, M, 0, 1, 6, 0, -1, 0, -2, 2, 0, -4, 0},
           /* S */
                      \{1, 0, 0, 0, 0, -3, 1, -1, -1, 0, 0, -3, -2, 1, M, 1, -1, 0, 2, 1, 0, -1, -2, 0, -3, 0\},\
/* T */
                      { 1, 0,-2, 0, 0,-3, 0,-1, 0, 0, 0,-1,-1, 0, M, 0,-1,-1, 1, 3, 0, 0,-5, 0,-3, 0},
           /* U */
                      /* V */
                      {0,-2,-2,-2,-1,-1,-2, 4, 0,-2, 2, 2,-2,_M,-1,-2,-2,-1, 0, 0, 4,-6, 0,-2,-2},
           /* W */
                      {-6,-5,-8,-7,-7, 0,-7,-3,-5, 0,-3,-2,-4,-4,_M,-6,-5, 2,-2,-5, 0,-6,17, 0, 0,-6},
           /* X */
                      /* Y */
                      {-3,-3, 0,-4,-4, 7,-5, 0,-1, 0,-4,-1,-2,-2,_M,-5,-4,-4,-3,-3, 0,-2, 0, 0,10,-4},
           1* Z *1
                      { 0, 1,-5, 2, 3,-5, 0, 2,-2, 0, 0,-2,-1, 1,_M, 0, 3, 0, 0, 0, 0, 0,-2,-6, 0,-4, 4}
   40
           };
```

45

25

50

FIGURE 4B

```
*/
             #include < stdio.h>
      5
             #include < ctype h>
             #define MAXJMP
                                         16
                                                   /* max jumps in a diag */
             #define MAXGAP
                                         24
                                                  /* don't continue to penalize gaps larger than this */
             #define JMPS
                                         1024
                                                  /* max jmps in an path */
    10
            #define MX
                                         4
                                                  /* save if there's at least MX-1 bases since last imp */
            #define DMAT
                                         3
                                                  /* value of matching bases */
            #define DMIS
                                         0
                                                  /* penalty for mismatched bases */
            #define DINSO
                                         8
                                                  /* penalty for a gap */
    15
            #define DINS1
                                         1
                                                  /* penalty per base */
            #define PINSO
                                         8
                                                  /* penalty for a gap */
            #define PINS1
                                                  /* penalty per residue */
            struct jmp {
    20
                      short
                                         n[MAXJMP];
                                                            /* size of jmp (neg for dely) */
                      unsigned short
                                         x[MAXJMP];
                                                            /* base no of imp in seq x */
};
                                                            /* limits seq to 2^16 -1 */
            struct diag {
    25
                     int
                                        score,
                                                            /* score at last jmp */
                     long
                                        offset;
                                                            /* offset of prev block */
                     short
                                        ŋmp;
                                                            /* current jmp index */
                     struct jmp
                                                           /* list of imps */
                                        Jp;
ļ.
            };
ī.
   30
            struct path {
27
                                                  /* number of leading spaces */
                     int
[]
                     short
                               n[JMPS];/* size of jmp (gap) */
                               x[JMPS],/* loc of jmp (last elem before gap) */
                     int
¼ 4 3 5
            };
char
                               *ofile;
/* output file name */
            char
                               *namex[2];
                                                           /* seq names getseqs() */
ļ
            char
                               *prog;
                                                           /* prog name for err msgs */
   40
           char
                               *seqx[2];
                                                           /* seqs getseqs() */
           int
                               dmax;
                                                           /* best diag: nw() */
           int
                               dmax0;
                                                           /* final diag */
           int
                              dna;
                                                           /* set if dna: main() */
           int
                              endgaps;
                                                           /* set if penalizing end gaps */
   45
           int
                              gapx, gapy;
                                                           /* total gaps in seqs */
           int
                              len0, len1;
                                                           /* seq lens */
           int
                              ngapx, ngapy;
                                                           /* total size of gaps */
           int
                              smax;
                                                           /* max score nw() */
           int
                              *xbm;
                                                           /* bitmap for matching */
   50
           long
                              offset:
                                                           /* current offset in imp file */
                    diag
           struct
                              *đx;
                                                           /* holds diagonals */
           struct
                     path
                              pp[2];
                                                           /* holds path for seqs */
           char
                              *calloc(), *malloc(), *index(), *strcpy();
   55
           char
                              *getseq(), *g_calloc();
```

FIGURE 4C

```
/* Needleman-Wunsch alignment program
             * usage: progs file1 file2
      5
                where file1 and file2 are two dna or two protein sequences.
                The sequences can be in upper- or lower-case an may contain ambiguity
                Any lines beginning with ';', '>' or '<' are ignored
                Max file length is 65535 (limited by unsigned short x in the jmp struct)
                A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
    10
                Output is in the file "align.out"
             * The program may create a tmp file in /tmp to hold info about traceback.
             * Original version developed under BSD 4.3 on a vax 8650
             */
    15
            #include "nw h"
            #include "day h"
            static
                       dbval[26] = {
                      1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
20
            };
                      pbval[26] = {
            static
13
                      1, 2[(1 < < ('D'-'A'))](1 < < ('N'-'A')), 4, 8, 16, 32, 64,
                      128, 256, 0xFFFFFFF, 1 < < 10, 1 < < 11, 1 < < 12, 1 < < 13, 1 < < 14.
    25
                      1 < < 15, 1 < < 16, 1 < < 17, 1 < < 18, 1 < < 19, 1 < < 20, 1 < < 21, 1 < < 22,
W
                      1 < < 23, 1 < < 24, 1 < < 25  (1 < < ('E'-'A'))  (1 < < ('Q'-'A'))
ļā
            };
TŲ.
            main(ac, av)
                                                                                                                              main
30
                               ac;
                      char
                               *av∏,
įä
                     prog = av[0];
if (ac != 3) {
13 35
                               fprintf(stderr,"usage: %s file1 file2\n", prog);
                               fprintf(stderr,"where file1 and file2 are two dna or two protein sequences \n").
ļ.ā
                               fprintf(stderr, "The sequences can be in upper- or lower-case\n");
                               fprintf(siderr, "Any lines beginning with ';' or '<' are ignored\n");
                               fprintf(stderr,"Output is in the file \"align out\"\n");
   40
                               exit(1);
                     namex[0] = av[1];
                     namex[1] = av[2];
                     seqx[0] = getseq(namex[0], \&len0),
   45
                     seqx[1] = getseq(namex[1], &len1);
                     \lambda bm = (dna)?_dbval: pbval;
                     endgaps = 0;
                                                            /* I to penalize endgaps */
                     ofile = "align.out";
                                                            /* output file */
   50
                     nw();
                                        /* fill in the matrix, get the possible imps */
                     readjmps();
                                        /* get the actual jmps */
                     print();
                                        /* print stats, alignment */
   55
                     cleanup(0);
                                        /* unlink any tmp files */
           }
```

FIGURE 4D

```
/* do the alignment, return best score: main()
             * dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
             * pro: PAM 250 values
      5
             * When scores are equal, we prefer mismatches to any gap, prefer
             * a new gap to extending an ongoing gap, and prefer a gap in seqx
             * to a gap in seq y.
             * f
            nw()
                                                                                                                                    nw
    10
                      char
                                          *px, *py;
                                                              /* segs and ptrs */
                      int
                                          *ndely, *dely;
                                                              /* keep track of dely */
                      int
                                          ndelx, delx;
                                                              /* keep track of delx */
                      int
                                          *tmp;
                                                              /* for swapping row0, row1 */
    15
                      int
                                                              /* score for each type */
                                          mis;
                      int
                                          ins0, ins1,
                                                              /* insertion penalties */
                      register
                                          id;
                                                              /* diagonal index */
                      register
                                          ij,
                                                              /* imp index */
                                          *col0, *col1;
                      register
                                                              /* score for curr, last row */
    20
                      register
                                                              /* index into seqs */
                                          xx, yy;
13
1 25 25
                      dx = (struct diag *)g_calloc("to get diags", len0+len1+1, sizeof(struct diag));
                      ndely = (int *)g calloc("to get ndely", len1+1, sizeof(int));
                      dely = (int *)g calloc("to get dely", len1+1, sizeof(int)),
<u>į.</u>
                      col0 = (int *)g calloc("to get col0", len1 + 1, sizeof(mt)),
                      col1 = (int *)g_calloc("to get col1", len1+1, sizeof(int)),
10
                      ins0 = (dna)^{9} DINS0. PINSO,
i.e.
                      ins1 = (dna)? DINS1 PINS1;
.
N<sub>30</sub>
62
                      smax = -10000;
if (endgaps) {
                                for (col0[0] = dely[0] = -ins0, yy = 1, yy < = len1; yy + +) {
who Said Hall
                                          col0[yy] = dely[yy] = col0[yy-1] - ins1;
    35
                                          ndely[yy] = yy,
                                col0[0] = 0;
                                                   /* Waterman Bull Math Biol 84 */
<u>ļ</u>.ā
                      }
                      else
    40
                                for (yy = 1; yy \le len1; yy++)
                                         dely[yy] = -ins0;
                      /* fill in match matrix
   45
                      for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
                                /* initialize first entry in col
                                */
                               if (endgaps) {
                                         if (xx = = 1)
   50
                                                   coll[0] = delx = -(ins0 + ins1);
                                         else
                                                   coll[0] = delx = col0[0] - ins1;
                                         ndelx = xx;
   55
                               else {
                                         coll[0] = 0;
                                         delx = -ins0;
                                         ndelx = 0;
                               }
```

Page 2 of nw.c

60

FIGURE 4E

 \dots nw for $(py = seqx[1], yy = 1; yy <= len1; py++, yy++) {$ mis = col0[yy-1];5 if (dna) mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;else $mis + = _day[*px-'A'][*py-'A'];$ 10 /* update penalty for del in x seq; * favor new del over ongong del * ignore MAXGAP if weighting endgaps if (endgaps | | ndely[yy] < MAXGAP) { 15 if (col0[yy] - ins0 > = dely[yy]) { dely[yy] = col0[yy] - (ins0 + ins1);ndely[yy] = 1;} else { dely[yy] -= insl;20 ndely[yy] + +;} else { if $(col0[yy] - (ins0 + ins1) > = dely[yy]) {$ dely[yy] = col0[yy] - (ins0 + ins1),25 ndely[yy] = 1,} else ndely[yy] + +, ļ. } F 30 /* update penalty for del in y seq; THE WA * favor new del over ongong del */ if (endgaps | | ndelx < MAXGAP) { if (coll[yy-1] - ins0 > = delx) { . [] 35 delx = coll[yy-1] - (ms0 + ms1),ndelx = 1;} else { Į.A delx -= insl;ndelx + +;40 } } else { if $(col1[yy-1] - (ins0 + ins1) > = delx) {$ delx = coll[yy-1] - (ins0 + ins1);ndelx = 1;45 } else ndelx++;} /* pick the maximum score; we're favoring 50 * mis over any del and delx over dely

60

55

55

FIGURE 4F

...bw

```
id = xx - yy + lenl - 1;
                                                                                                      if (mis > = delx && mis > = dely[yy])
               5
                                                                                                                              coll[yy] = mis;
                                                                                                      else if (delx > = dely[yy]) {
                                                                                                                              coll[yy] = delx;
                                                                                                                              ij = dx[id].ijmp;
                                                                                                                              if (dx[id].jp.n[0] && (!dna | | (ndelx > = MAXJMP))
          10
                                                                                                                              && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINSO)) {
                                                                                                                                                      dx[id] ijmp++;
                                                                                                                                                      if (++ij > = MAXJMP) {
                                                                                                                                                                              writejmps(id);
                                                                                                                                                                              ij = dx[id] ijmp = 0;
          15
                                                                                                                                                                              dx[id] offset = offset;
                                                                                                                                                                              offset += sizeof(struct jmp) + sizeof(offset);
                                                                                                                                                      }
                                                                                                                              dx[id] jp n[ij] = ndelx;
          20 -
                                                                                                                              dx[id] jp x[ij] = xx;
                                                                                                                              dx[id] score = delx,
                                                                                                      }
The state when the state state
                                                                                                      else {
                                                                                                                              coll[yy] = dely[yy],
         25
                                                                                                                              ij = dx[id] ijmp,
                                                      if (dx[id] \text{ jp } n[0] \&\& (!dna | | (ndely[yy]) > = MAXJMP
                                                                                                                              && xx > dx[id] yp x[ij]+MX) || mis > dx[id] score+DINS0)) {
                                                                                                                                                      dx[id] ijmp++,
          30
                                                                                                                                                      if (++ij > = MAXJMP) {
                                                                                                                                                                              writejmps(id).
M. Hote M.
                                                                                                                                                                              ij = dx[id] ijmp = 0,
                                                                                                                                                                              dx[id] offset = offset,
                                                                                                                                                                              offset += sizeof(struct jmp) + sizeof(offset);
          35
Mar Alle Mar
                                                                                                                                                     }
                                                                                                                             dx[id] jp n[ij] = -ndely[yy];
                                                                                                                             dx[id] yp.x[y] = xx;
                                                                                                                              dx[id].score = dely[yy];
          40
                                                                                                     if (xx == len0 && yy < len1) {
                                                                                                                             /* last col
                                                                                                                               */
                                                                                                                             if (endgaps)
         45
                                                                                                                                                     coll[yy] = ins0 + ins1*(len1-yy);
                                                                                                                             if (coll[yy] > smax) {
                                                                                                                                                     smax = coll[yy];
                                                                                                                                                     dmax = id;
                                                                                                                             }
         50
                                                                                                     }
                                                                             if (endgaps && xx < len0)
                                                                                                     coll[yy-1] = ins0 + ins1*(len0-xx);
                                                                             if (coll[yy-1] > smax) {
        55
                                                                                                     smax = coll[yy-1];
                                                                                                     dmax = id;
                                                                            tmp = col0; col0 = col1; col1 = tmp;
        60
                                                     (void) free((char *)ndely);
                                                     (void) free((char *)dely);
                                                     (void) free((char *)col0);(void) free((char *)col1);}
```

Page 4 of nw.c

FIGURE 4G

```
1*
             * print() -- only routine visible outside this module
      5
             * getmat() -- trace back best path, count matches: print()
             * pr_align() -- print alignment of described in array p[]: print()
             * dumpblock() -- dump a block of lines with numbers, stars: pr_align()
    10
             * nums() - put out a number line: dumpblock()
             * putline() -- put out a line (name, [num], seq, [num]): dumpblock()
             * stars() - -put a line of stars: dumpblock()
             * stripname() -- strip any path and prefix from a seqname
    15
            #include "nw h"
            #define SPC
            #define P LINE 256
                                         /* maximum output line */
    20
            #define P SPC
                                         /* space between name or num and seq */
            extern
                      day[26][26];
int
                      olen;
                                         /* set output line length */
            FILE
                      *fx,
                                         /* output file */
    25
            print()
                                                                                                                               print
                     int
                               Ix, Iy, firstgap, lastgap;
                                                            /* overlap */
Q
= 30
                     if ((fx = fopen(ofile, "w")) = = 0) {
P.
                               fprintf(stderr," %s can't write %s\n", prog, ofile);
                               cleanup(1);
22
fprintf(fx, " < first sequence: %s (length = %d)\n", namex[0], len0);
   35
                     fprintf(fx, " < second sequence: %s (length = %d)\n", namex[1], len1);
olen = 60;
                     lx = len0,
                     ly = len1;
                     firstgap = lastgap = 0;
   40
                     if (dmax < len1 - 1) {
                                                  /* leading gap in x */
                               pp[0].spc = firstgap = len1 - dmax - 1;
                               ly -= pp[0] spc;
                     else if (dmax > len1 - 1) { /* leading gap in y */
   45
                               pp[1] spc = firstgap = dmax - (len1 - 1);
                               lx -= pp[1].spc;
                     if (dmax0 < len0 - 1) {
                                                 /* trailing gap in x */
                              lastgap = len0 - dmax0 - 1;
   50
                              lx -= lastgap;
                     else if (dmax0 > len0 - 1) { /* trailing gap in y */
                              lastgap = dmax\theta - (len0 - 1);
                              ly -= lastgap;
   55
                    getmat(lx, ly, firstgap, lastgap);
                    pr align();
           }
```

FIGURE 4H

```
* trace back the best path, count matches
      5
             static
             getmat(lx, ly, firstgap, lastgap)
                                                                                                                        getmat
                      int
                               lx, ly;
                                                           /* "core" (minus endgaps) */
                      int
                               firstgap, lastgap;
                                                           /* leading trailing overlap */
             {
    10
                      int
                                        nm, 10, i1, siz0, siz1;
                      char
                                        outx[32];
                      double
                                        pct;
                      register
                                        n0, n1;
                      register char
                                        *p0, *p1;
    15
                      /* get total matches, score
                      */
                      i0 = i1 = siz0 = siz1 = 0;
                      p0 = seqx[0] + pp[1] spc;
    20
                     p1 = seqx[1] + pp[0] spc;
                      n0 = pp[1].spc + 1;
                     nl = pp[0].spc + 1;
nm = 0;
    25
                      while ( *p0 && *p1 ) {
                               if (siz0) {
                                        pl++;
                                        nl++,
q
                                        s120--;
i.a
    30
else if (siz1) {
                                        p0++;
                                        n0++;
113
                                        SIZ1--,
    35
else {
                                       if (xbm[*p0-'A']&xbm[*p1-'A'])
                                                 nm++;
                                       if (n0++=pp[0],x[n0])
    40
                                                 siz0 = pp[0].n[i0++];
                                       if (n1 + + = pp[1].x[1])
                                                 siz1 = pp[1].n[il++];
                                       p0++;
                                       p1++;
    45
                              }
                     }
                     /* pct homology:
                      * if penalizing endgaps, base is the shorter seq
    50
                      * else, knock off overhangs and take shorter core
                      */
                     if (endgaps)
                              lx = (len0 < len1)? len0 : len1;
                     else
    55
                              lx = (lx < ly)^2 lx : ly;
                     pct = 100.*(double)nm/(double)lx;
                     fprintf(fx, "\n");
                     fprintf(fx, " < %d match%s in an overlap of %d: %.2f percent similarity\n",
                              nm, (nm = = 1)? "" : "es", lx, pct);
   60
```

E

```
fprintf(fx, " < gaps in first sequence: %d", gapx);
                                                                                                                        ...getmat
                      if (gapx) {
      5
                               (void) sprintf(outx, " (%d %s%s)",
                                         ngapx, (dna)? "base": "residue", (ngapx = = 1)? "": "s");
                               fprintf(fx, "%s", outx);
                      fprintf(fx, ", gaps in second sequence: %d", gapy);
    10
                      if (gapy) {
                               (void) sprintf(outx, " (%d %s%s)",
                                         ngapy, (dna)? "base": "residue", (ngapy == 1)? "": "s");
                               fprintf(fx, "%s", outx);
    15
                     if (dna)
                               fprintf(fx,
                               "n < score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)n",
                               smax, DMAT, DMIS, DINSO, DINSI);
                      else
    20
                               fprintf(fx,
                                "\n < score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
                               smax, PINS0, PINS1),
if (endgaps)
                               fprintf(fx,
    25
                                "<endgaps penalized left endgap: %d %s%s, right endgap: %d %s%s\n",
                               firstgap, (dna)? "base": "residue", (firstgap = = 1)? "". "s",
                               lastgap, (dna)? "base". "residue", (lastgap = = 1)? "". "s");
Ŀå
                     else
fprintf(fx, " < endgaps not penalized\n");
<u>‡</u> 30
            }
TŲ.
             static
                               nm;
                                                  /* matches in core -- for checking */
Į.
             static
13
                               Imax;
                                                  /* lengths of stripped file names */
             static
                               ij[2],
                                                  /* jmp index for a path */
   35
             static
                               nc[2],
                                                  /* number at start of current line */
į.
             static
                               ni[2];
                                                  /* current elem number -- for gapping */
static
                               siz[2];
             static char
                               *ps[2];
                                                  /* ptr to current element */
             static char
                               *po[2];
                                                  /* ptr to next output char slot */
    40
             static char
                               out[2][P LINE];
                                                 /* output line */
            static char
                               star[P LINE];
                                                  /* set by stars() */
             * print alignment of described in struct path pp[]
   45
            */
            static
            pr_align()
                                                                                                                        pr align
                     int
                                                  /* char count */
                                        nn;
   50
                     int
                                        more;
                     register
                                        ì;
                     for (i = 0, lmax = 0; i < 2; i++) {
                              nn = stripname(namex[i]);
   55
                              if (nn > lmax)
                                        lmax = nn;
                              nc[i] = 1;
                              m[i] = 1;
   60
                              siz[i] = ij[i] = 0;
                              ps[i] = seqx[i];
                              po[i] = out[i];
                                                                                                 Page 3 of nwprint.c
```

FIGURE 4J

```
for (nn = nm = 0, more = 1; more;) {
                                                                                                               ...pr align
                              for (i = more = 0; i < 2; i++) {
     5
                                       * do we have more of this sequence?
                                      if (!*ps[i])
                                               continue;
    10
                                      more++;
                                      if (pp[i] spc) { /* leading space */
                                               *po[i]++='';
    15
                                               pp[1].spc--;
                                      *po[i]++= '-';
                                               S1Z[1]--;
    20
                                      }
                                      else {
                                                        /* we're putting a seq element
*po[i] = *ps[i];
                                               if (islower(*ps[1]))
   25
                                                        *ps[i] = toupper(*ps[i]);
                                               po[i]++;
                                               ps[i]++;
.
<u>|</u> 30
                                                * are we at next gap for this seq?
* /
                                               if (m[i] = pp[i] \times [n[i]) {
ŝ
35
                                                        * we need to merge all gaps
                                                        * at this location
<u>La</u>
siz[i] = pp[i] n[ij[i] + +];
                                                        while (ni[i] = pp[i].x[ij[i]])
                                                                 siz[i] + = pp[i].n[ij[i] + +];
<sup>1</sup> 40
                                               }
                                               m[i]++;
                             if (++nn = = olen | | !more && nn) {
   45
                                      dumpblock();
                                      for (i = 0, i < 2; i++)
                                              po[i] = out[i];
                                     nn = 0;
                             }
   50
                   }
           }
           * dump a block of lines, including numbers, stars: pr_align()
   55
           */
           static
           dumpblock()
                                                                                                             dumpblock
                   register i;
  60
                   for (i = 0; i < 2; i++)
                            *po[i]-- = '\0';
                                                                                           Page 4 of nwprint.c
```

FIGURE 4K

```
...dumpblock
                                                                     (void) putc('\n', fx);
                    5
                                                                     for (i = 0; i < 2; i++) {
                                                                                                  if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
                                                                                                                               if (i = 0)
                                                                                                                                                          nums(i);
                                                                                                                              if (i == 0 && *out[1])
              10
                                                                                                                                                          stars();
                                                                                                                              putline(i);
                                                                                                                              if (i == 0 \&\& *out[1])
                                                                                                                                                          fprintf(fx, star);
                                                                                                                              if (i = = 1)
              15
                                                                                                                                                          nums(i);
                                                                                                 }
                                                                    }
                                       }
              20
THE CHARGE AND A STREET WAS AND ASSESSMENT ASSESSMENT AND ASSESSMENT ASSESSMENT ASSESSMENT AND ASSESSMENT ASSESSM
                                         * put out a number line: dumpblock()
                                       static
                                       nums(ix)
                                                                                                                                                                                                                                                                                                                                                                                            nums
              25
                                                                    int
                                                                                                                             /* index in out[] holding seq line */
                                                                    char
                                                                                                                              nline[P_LINE],
                                                                                                                            i, j;
                                                                    register
30 35 35
                                                                    register char
                                                                                                                              *рл, *рх, *ру;
                                                                  for (pn = nline, i = 0; i < lmax + P_SPC; i++, pn++)
                                                                                                *pn = ' ';
                                                                  for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
                                                                                               if (*py == ' ` | | *py == '-')
*pn = ' ';
                                                                                                else {
                                                                                                                            if (i\%10 == 0) || (i == 1 \&\& nc[ix]!= 1)) {
                                                                                                                                                       j = (i < 0)? -i : i;
                                                                                                                                                        for (px = pn; j; j /= 10, px--)
            40
                                                                                                                                                                                      *px = j\%10 + '0';
                                                                                                                                                        if (i < 0)
                                                                                                                                                                                      *px = '-';
                                                                                                                            }
                                                                                                                           else
           45
                                                                                                                                                        *pn = ' ';
                                                                                                                           i++;
                                                                                              }
                                                                 *pn = '0';
          50
                                                                 nc[ix] = i;
                                                                 for (pn = nline; *pn; pn++)
                                                                                            (void) putc(*pn, fx);
                                                                 (void) putc('\n', fx);
                                   }
         55
                                     * put out a line (name, [num], seq, [num]): dumpblock()
                                  static
         60
                                 putline(ix)
                                                                                                                                                                                                                                                                                                                                                                                  putline
                                                              int
                                                                                            ix;
                                  ₹
                                                                                                                                                                                                                                                                         Page 5 of nwprint.c
```

FIGURE 4L

```
...putline
                                        ĩ;
      5
                      register char
                                        *px;
                      for (px = namex[ix], i = 0; *px && *px != ':'; px++, i++)
                               (void) putc(*px, fx);
                      for (; i < lmax+P SPC; i++)
    10
                               (void) putc(' ', fx);
                      /* these count from 1:
                      * no is current element (from 1)
                       * nc[] is number at start of current line
    15
                      for (px = out[ix]; *px; px++)
                               (void) putc(*px&0x7F, fx);
                      (void) putc('\n', fx);
             }
    20
* put a line of stars (seqs always in out[0], out[1]): dumpblock()
    25
            static
             stars()
                                                                                                                          stars
             {
                      int
                                        *p0, *p1, cx, *px;
                      register char
    30
                      if (!*out[0] | (*out[0] = " ` & & *(po[0]) = = " ") | )
!*out[1] | (*out[1] == ``&& *(po[1]) == ``))
                              return:
                     px = star;
    35
                     for (i = lmax + P_SPC; i; i-)
                               *px++ = ' ';
14
                     for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
                              if (isalpha(*p0) && isalpha(*p1)) {
    40
                                        if (xbm[*p0-'A']&xbm[*p1-'A']) {
                                                 \epsilon x = '*';
                                                 nm++;
    45
                                        else if (!dna && _day[*p0-'A'][*p1-'A'] > 0)
                                                 cx = '.';
                                        else
                                                 cx = ' ';
                              }
    50
                              else
                                       cx =
                              *px++=cx;
                     *px + + = '\n';
    55
                     *px = '0';
            }
```

FIGURE 4M

```
* strip path or prefix from pn, return len: pr_align()
      5
           static
            stripname(pn)
                                                                                                           stripname
                             *pn;
                                     /* file name (may be path) */
            {
                    register char
                                      *px, *py;
    10
                    py = 0;
                    for (px = pn; *px; px++)
                             if (*px = T)
                                     py = px + 1;
    15
                    if (py)
                             (void) strcpy(pn, py);
                    return(strlen(pn));
           }
    20
40
   45
   50
   55
   60
```

FIGURE 4N

```
* cleanup() - cleanup any tmp file
              * getseq() -- read in seq, set dna, len, maxlen
      5
              * g_calloc() -- calloc() with error checkin
              * readjmps() -- get the good jmps, from tmp file if necessary
              * writejmps() -- write a filled array of jmps to a tmp file: nw()
             #include "nw h"
    10
             #include < sys/file h>
             char
                       *jname = "/tmp/homgXXXXXX";
                                                                      /* tmp file for imps */
             FILE
    15
             int
                      cleanup();
                                                                      /* cleanup tmp file */
             long
                      lseek();
             * remove any tmp file if we blow
    20
             */
             cleanup(1)
                                                                                                                          cleanup
                                ì,
if (fi)
    25
                                (void) unlink(jname);
                      exit(i);
             }
30
             * read, return pir to seq, set dna, len, maxlen
             * skip lines starting with ';', '<', or '>'
* seq in upper or lower case
             */
            char
    35
            getseq(file, len)
                                                                                                                            getseq
                                *file;
                      char
                                         /* file name */
                                *len;
                      int
                                         /* seq len */
                      char
                                         line[1024], *pseq;
    40
                      register char
                                         *px, *py;
                      int
                                         natge, tlen;
                      FILE
                     if ((fp = fopen(file, "r")) == 0) {
    45
                               fprintf(stderr, "%s: can't read %s\n", prog, file);
                     }
                     then = natgc = 0;
                     while (fgets(line, 1024, fp)) {
   50
                               if (*line == ';' ]) *line == '<' || *line == '>')
                                        continue;
                               for (px = line; *px != '\n'; px++)
                                        if (isupper(*px) | | islower(*px))
                                                  ilen++;
   55
                     if ((pseq = malloc((unsigned)(tlen+6))) = = 0) {
                               fprintf(stderr," %s: malloc() failed to get %d bytes for %s\n", prog, tlen+6, file);
   60
                     pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';
```

```
...getseq
                                                             py = pseq + 4;
                                                             *len = tlen;
                 5
                                                             rewind(fp),
                                                              while (fgets(line, 1024, fp)) {
                                                                                      if (*line == ',' || *line == '<' || *line == '>')
                                                                                                                 continue;
           10
                                                                                      for (px = line; *px != '\n'; px + +) {
                                                                                                                 if (isupper(*px))
                                                                                                                                           *py++ = *px;
                                                                                                                 else if (islower(*px))
                                                                                                                                           *py++ = toupper(*px);
           15
                                                                                                                 if (index("ATGCU",*(py-1)))
                                                                                                                                           natgc++;
                                                                                       }
                                                              *py++ = '\0',
           20
                                                              *py = '0';
The second of th
                                                             (void) fclose(fp);
                                                             dna = natgc > (tlen/3);
                                                             return(pseq +4),
                                   }
           25
                                   char
                                                                                                                                                                                                                                                                                                                                             g_calloc
ļA
                                   g_calloc(msg, nx, sz)
                                                                                                                                           /* program, calling routine */
T.U
                                                             char
                                                                                      *msg,
                                                                                                                                           /* number and size of elements */
                                                                                      nx, sz,
           30
char
                                                                                                                 *px, *calloc();
                                                             if ((px = calloc((unsigned)nx, (unsigned)sz)) = = 0) {
           35
                                                                                                                fprintf(stderr, "%s: g calloc() failed %s (n= %d, sz = %d)\n", prog, msg, nx, sz);
                                                            return(px),
           40
                                   * get final jmps from dx[] or tmp file, set pp[], reset dmax: main()
           45
                                                                                                                                                                                                                                                                                                                                     readjmps
                                  readjmps()
                                                                                                                fd = -1;
                                                            int
                                                            int
                                                                                                                siz, i0, 11;
                                                            register i, j, xx;
          50
                                                           if (fj) {
                                                                                      (void) fclose(fj);
                                                                                     if ((fd = opén(jname, O RDONLY, 0)) < 0) {
                                                                                                               fprintf(stderr, "%s: can't open() %s\n", prog, jname);
          55
                                                           for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; ; i++) {
                                                                                     while (1) {
          60
                                                                                                               for (j = dx[dmax].ijmp; j > = 0 && dx[dmax] jp x[j] > = xx; j--)
                                                                                                                                                                                                                                                                           Page 2 of nwsubr.c
```

FIGURE 4P

```
...readjmps
                                        if (j < 0 \&\& dx[dmax]).offset && fj) {
                                                  (void) Iseek(fd, dx[dmax] offset, 0);
      5
                                                  (void) read(fd, (char *)&dx[dmax] jp, sizeof(struct jmp));
                                                  (void) read(fd, (char *)&dx[dmax] offset, sizeof(dx[dmax].offset));
                                                  dx[dmax].iimp = MAXJMP-1;
                                        }
                                        else
    10
                                                  break;
                               if (i > = JMPS) {
                                        fprintf(stderr, "%s: too many gaps in alignment\n", prog);
                                        cleanup(1);
    15
                               if (i > = 0) {
                                        siz = dx[dmax] p.n[j];
                                        xx = dx[dmax] jp x[j],
                                        dmax += siz;
    20
                                        if (siz < 0) {
                                                                    /* gap in second seq */
                                                  pp[1] n[i1] = -siz;
                                                 xx + = siz,
13
13
13
25
                                                 /* id = xx - yy + len1 - 1
pp[1] x[i1] = xx - dmax + len1 - 1;
                                                 gapy + +,
10
                                                 ngapy -= siz;
            /* ignore MAXGAP when doing endgaps */
l.s.
   30
                                                 siz = (-siz < MAXGAP | | endgaps)^{7} - siz : MAXGAP;
ĨŲ
                                                 11++,
else if (siz > 0) { /* gap in first seq */
                                                 pp[0] n[i0] = siz,
    35
                                                 pp[0] x[i0] = xx;
gapx++;
                                                 ngapx += siz,
            /* ignore MAXGAP when doing endgaps */
                                                 siz = (siz < MAXGAP | endgaps)? siz : MAXGAP;
    40
                                        }
                              }
                              else
                                        break;
   45
                     }
                     /* reverse the order of imps
                      */
                     for (j = 0, i0--; j < i0; j++, i0--)
   50
                              i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0] n[i0] = i;
                              i = pp[0].x[j]; pp[0].x[j] = pp[0] x[i0]; pp[0] x[i0] = i;
                     for (j = 0, i1--; j < i1; j++, i1--) {
                              i = pp[1].n[j]; pp[1] n[j] = pp[1].n[i]; pp[1].n[i] = i;
   55
                              i = pp[1].x[j], pp[i] x[j] = pp[i].x[ii]; pp[i].x[ii] = i;
                     if (fd > = 0)
                              (void) close(fd);
                     if (fj) {
   60
                              (void) unlink(iname);
                              f_1 = 0;
                              offset = 0;}
                                                                                               Page 3 of nwsubr.c
```

FIGURE 4Q

```
/*
             * write a filled jmp struct offset of the prev one (if any): nw()
      5
                                                                                                                  writejmps
            writejmps(ix)
                               ix;
                      char
                               *mktemp();
    10
                      if ('fj) {
                               if (mktemp(jname) < 0) {
                                        fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);
                                        cleanup(1);
    15
                               if ((f_j = fopen(jname, "w")) = = 0) {
                                        fprintf(stderr, "%s- can't write %s\n", prog, jname);
                                        exi(1);
                               }
    20
                      (void) fwrite((char *)&dx[ix] jp, sizeof(struct jmp), 1, fj),
                      (void) fwrite((char *)&dx[ix] offset, sizeof(dx[ix] offset), 1, fj);
            }
    25
30
1.4
    45
    50
    55
   60
```

GTGCTCTCCGAGGACAAGCAGGAGGAGGAGGTGGAGCTGGTGAAGCACCATCTGTGGGCTCTG
GAAGTGTGCTACATCTCAGCCTTGGTCTTGTCCTGCTTACTCACCTTCCTGGTCCTGATG
CGCTCACTGGTGACACAAGGACCAACCTTCGAGCTCTGCACCGAGGAGCTGCCCTGGAC
TTGAGTCCCTTGCATCGGAGTCCCCATCCCTCCGCCAAGCCATATTCTGTTGGATGAGC

TTCAGTGCCTACCAGACAGCCTTTATCTGCCTTGGGCTCCTGGTGCAGCAGATCATCTTC
TTCCTGGGAACCACGGCCCTGGCCTTCCTGGTGCTCATGCCTGGCAGGAAC
CTCCTGCTCTTCCGTTCCCTGGAGTCCTCGTGGCCTTCTGGCTGACTTTTGGCCCTGGCT
GTGATCCTGCAGAACATGGCAGCCCATTGGGTCTTCCTGGAGACTCATGATGGACACCCA
CAGCTGACCAACCGGCGAGTGCTCTATGCAGCCACCTTTCTTCTCTCCCCCTCAATGTG
CTGGTGGGTGCCATGGTGGCCACCTGGCGAGAGCCGCCACTCTCACAACGCCATC
CACCTTGGCCAGATGGACCTCAGCCTGCCGAGAGCCGCCACTCTCGACCCCGGC
TACTACACGTACCGAA

CACAACCAGCCACCCTCTAGGATCCCAGCCCAGCTGGTGCTGGGCTCAGAGGAGAAGGC 5 CCCGTGTTGGGAGCACCCTGCTTGCCTGGAGGGACAAGTTTCCGGGGAGAGATCAATAAAG GAAAGGAAAGAGACAAGGAAGGGAGAGGTCAGGAGAGCGCTTGATTGGAGGAGAAGGGCC AGAGA**ATG**TCGTCCCAGCCAGCAGGGAACCAGACCTCCCCGGGGCCACAGAGGACTACT 10 TGTCAATCCTTGTGCTGCTCCTGGCCATGCTGGTGAGGCGCCGCCAGCTCTGGCCTG ACTGTGTGCGTGGCAGGCCCGGCCTGCCCAGGCCCCGGGCAGTGCCTGCTGTTTTCA TGGTCCTCCTGAGCTCCCTGTGTTTGCTGCTCCCCGACGAGGACGCATTGCCCTTCCTGA CTCTCGCCTCAGCACCCAGCCAAGATGGGAAAACTGAGGCTCCAAGAGGGGCCTGGAAGA TACTGGGACTGTTCTATTATGCTGCCCTCTACTACCCTCTGGCTGCCTGTGCCACGGCTG GCCACACAGCTGCACACCTGCTCGGCAGCACGCTGTCCTGGGCCCACCTTGGGGTCCAGG 15. TCTGGCAGAGGGCAGAGTGCCCCAGGTGCCCAAGATCTACAAGTACTACTCCCTGCTGG CCTCCCTGCCTCCTGCTGGGCCTCGGATTCCTGAGCCTTTGGTACCCTGTGCAGCTGG TGAGAAGCTTCAGCCGTAGGACAGGAGCAGGCTCCAAGGGGCTGCAGAGCAGCTACTCTG AGGAATATCTGAGGAACCTCCTTTGCAGGAAGAAGCTGGGAAGCAGCTACCACACCTCCA 20 AGCATGGCTTCCTGTCCTGGGCCCGCGTCTGCTTGAGACACTGCATCTACACTCCACAGC CAGGATTCCATCTCCCGCTGAAGCTGGTGCTTTCAGCTACACTGACAGGGACGGCCATTT ACCAGGTGGCCCTGCTGCTGGTGGGCGTGGTACCCACTATCCAGAAGGTGAGGGCAG GGGTCACCACGGATGTCTCCTACCTGCTGGCCGGCTTTGGAATCGTGCTCTCCGAGGACA AGCAGGAGGTGGTGGAGCTGAAGCACCATCTGTGGGCTCTGGAAGTGTGCTACATCT 25 CAGCCTTGGTCTTGTCCTGCTTACTCACCTTCCTGGTCCTGATGCGCTCACTGGTGACAC ACAGGACCAACCTTCGAGCTCTGCACCGAGGAGCTGCCCTGGACTTGAGTCCCTTGCATC GGAGTCCCCATCCCTCCCGCCAAGCCATATTCTGTTGGATGAGCTTCAGTGCCTACCAGA CAGCCTTTATCTGCCTTGGGCTCCTGGTGCAGCAGATCATCTTCTTCCTGGGAACCACGG CCCTGGCCTTCCTGGTGCTCATGCCTGTGCTCCATGGCAGGAACCTCCTGCTCTTCCGTT CCCTGGAGTCCTCGTGGCCCTTCTGGCTGACTTTGGCCCTGGCTGTGATCCTGCAGAACA TGGCAGCCCATTGGGTCTTCCTGGAGACTCATGATGGACACCCACAGCTGACCAACCGGC TGGCCACCTGGCGAGTGCTCCTCTCTGCCCTCTACAACGCCATCCACCTTGGCCAGATGG ACCTCAGCCTGCCACCGAGAGCCGCCACTCTCGACCCCGGCTACTACACGTACCGAA 35 ACTTCTTGAAGATTGAAGTCAGCCAGTCGCATCCAGCCATGACAGCCTTCTGCTCCCTGC TCCTGCAAGCGCAGAGCCTCCTACCCAGGACCATGGCAGCCCCCCAGGACAGCCTCAGAC CAGGGGAGGAAGACGAAGGGATGCAGCTGCTACAGACAAAGGACTCCATGGCCAAGGGAG CTAGGCCCGGGGCCAGCCGCAGGGCTCGCTGGGGTCTGGCCTACACGCTGCTGCACA ACCCAACCCTGCAGGTCTTCCGCAAGACGCCCTGTTGGGTGCCAATGGTGCCCAGCCC**T** 40 **GA**GGCAGGGAAGGTCAACCCACCTGCCCATCTGTGCTGAGGCATGTTCCTGCCTACCAC GGATCACTGTGGTTGGGTGGAGGTCTGTCTGCACTGGGAGCCTCAGGAGGGCTCTGCTCC CCTTGGTCCAGGAGCCAGTTGAGCCAGGGCAGCCACATCCAGGCGTCTCCCTACCCTGGC 45 TCTGCCATCAGCCTTGAAGGGCCTCGATGAAGCCTTCTCTGGAACCACTCCAGCCCAGCT CCACCTCAGCCTTCACGCTGTGGAAGCAGCCAAGGCACTTCCTCACCCCCTCAG CGCCACGGACCTCTCTGGGGAGTGGCCGGAAAGCTCCCGGGCCTCTGGCCTGCAGGGCAG CCCAAGTCATGACTCAGACCAGGTCCCACACTGAGCTGCCCACACTCGAGAGCCAGATAT TTTTGTAGTTTTTATGCCTTTGGCTATTATGAAAGAGGTTAGTGTGTTCCCTGCAATAAA 50 CTTGTTCCTGAGAAAA

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MSSQPAGNQTSPGATEDYSYGSWYIDEPQGGEELQPEGEVPSCHTSIPPGLYHACLASL SILVLLLAMLVRRRQLWPDCVRGRPGLPRPRAVPAAVFMVLLSSLCLLLPDEDALPFL TLASAPSQDGKTEAPRGAWKILGLFYYAALYYPLAACATAGHTAAHLLGSTLSWAHLGV QVWQRAECPQVPKIYKYYSLLASLPLLLGLGFLSLWYPVQLVRSFSRRTGAGSKGLQSS YSEEYLRNLLCRKKLGSSYHTSKHGFLSWARVCLRHCIYTPQPGFHLPLKLVLSATLTG TAIYQVALLLLVGVVPTIQKVRAGVTTDVSYLLAGFGIVLSEDKQEVVELVKHHLWALE VCYISALVLSCLLTFLVLMRSLVTHRTNLRALHRGAALDLSPLHRSPHPSRQAIFCWMS FSAYQTAFICLGLLVQQIIFFLGTTALAFLVLMPVLHGRNLLLFRSLESSWPFWLTLAL AVILQNMAAHWVFLETHDGHPQLTNRRVLYAATFLLFPLNVLVGAIVATWRVLLSALYN AIHLGQMDLSLLPPRAATLDPGYYTYRNFLKIEVSQSHPAMTAFCSLLLQAQSLLPRTM AAPQDSLRPGEEDEGMQLLQTKDSMAKGARPGASRGRARWGLAYTLLHNPTLQVFRKTA LLGANGAQP

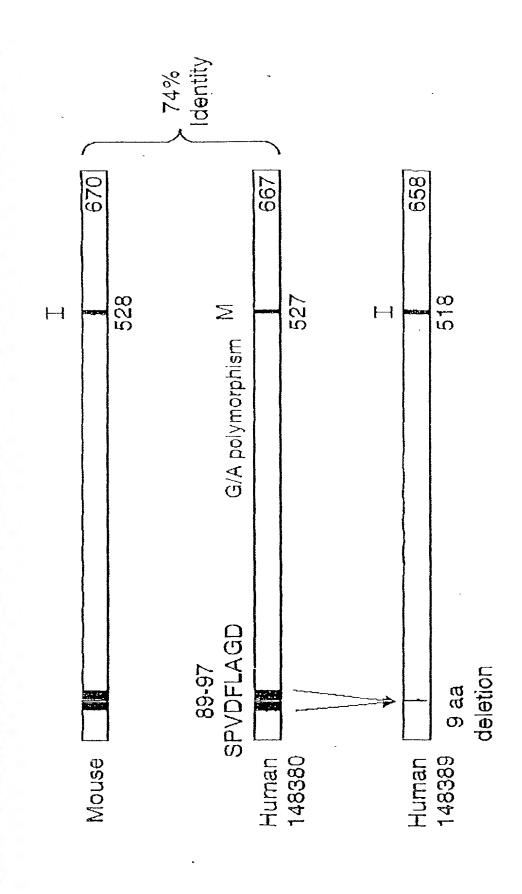
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Important features of the protein:
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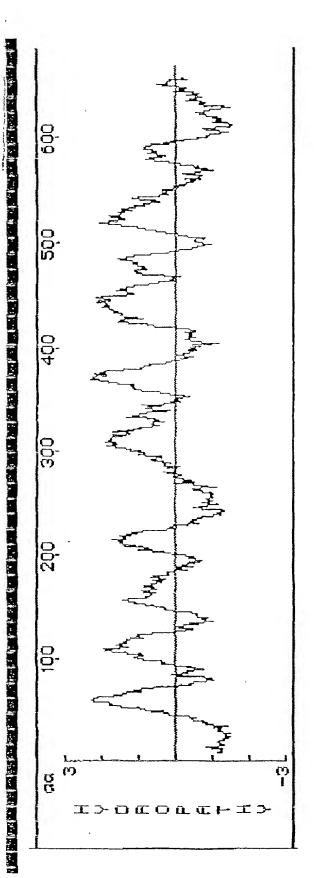
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IJ
        Signal peptide:
none
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        Transmembrane domain:
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        54 - 71
10
        93-111
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        140-157
11 25
        197-214
        291-312
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111
        356-371
        425-444
١,٠.]
        464-481
  30
        505-522
Motif name: N-glycosylation site.
ļA
              8-12
  35
       Motif name: N-myristoylation site.
             50-56
            167 - 173
  40
            232-238
            308-314
            332-338
            516-522
            618 - 624
  45
            622-628
            631-637
            652-658
       Motif name: Prokaryotic membrane lipoprotein lipid attachment
  50
       site.
            355-366
       Motif name: ATP/GTP-binding site motif A (P-loop).
  55
            123-131
```

Stra6 Variant Clones

"我是想到你,我们就是我们的人,我们也不会有一个人,我们也不是一个人,我们也不是一个人,我们也不会有一个人,我们也不会有一个人,我们也会会看到这一个人,我们也是

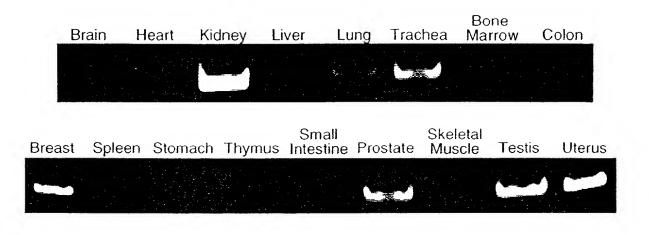


Hydrophobicity Plot of Human Stra6

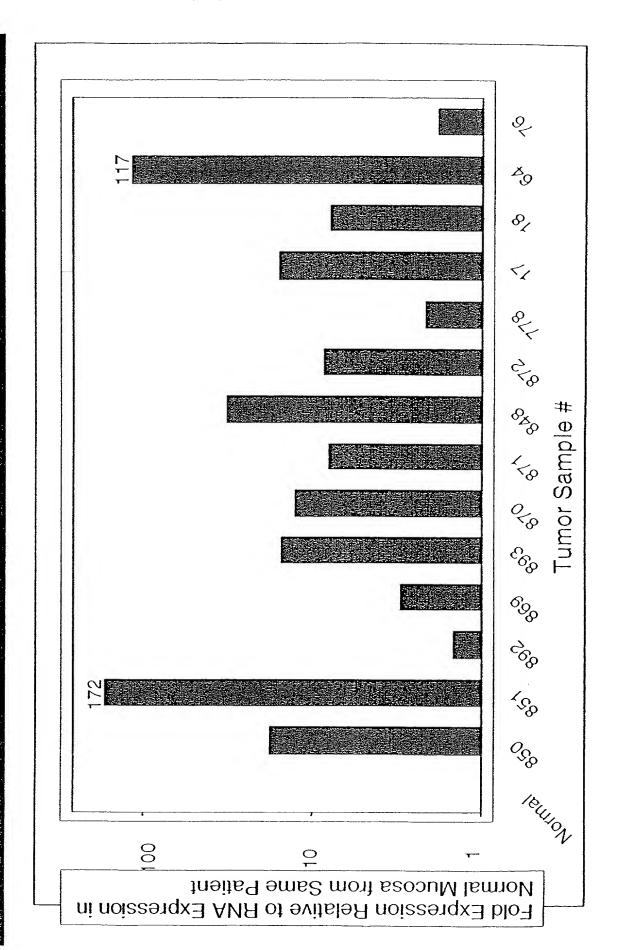




- 667 Amino Acids -->50% Residues Hydrophobic
 - 73.5 kDa Protein
- 9 Potential Transmembrane Domains



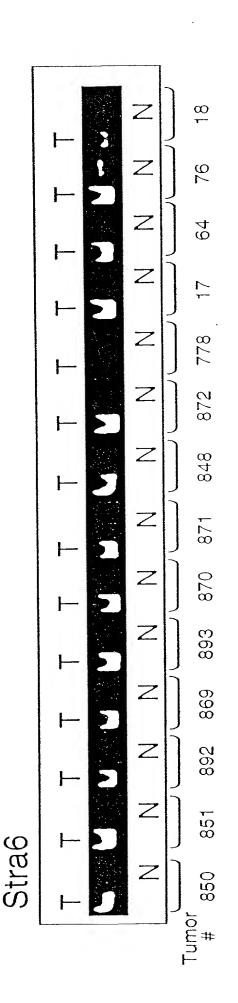
Stra6 RNA Expression in Human Colon Tumor Tissue



The state of the s

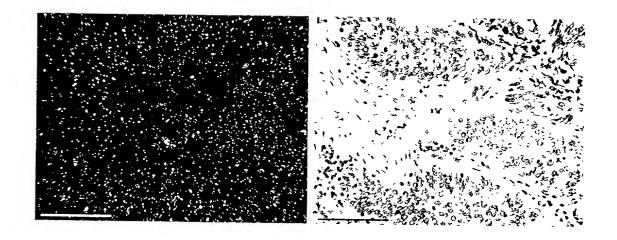
Tissue vs Normal Mucosa From the Same Patient Stra6 RNA Expression in Human Colon Tumor

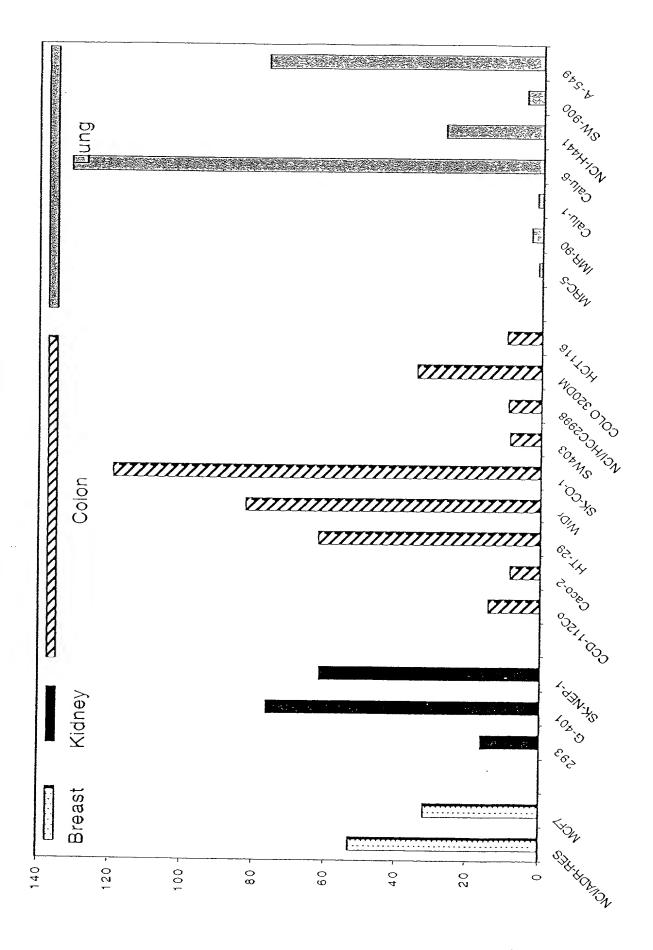
Taqman Product Analysis After 40 Cycles



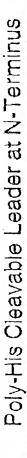
GAPDH

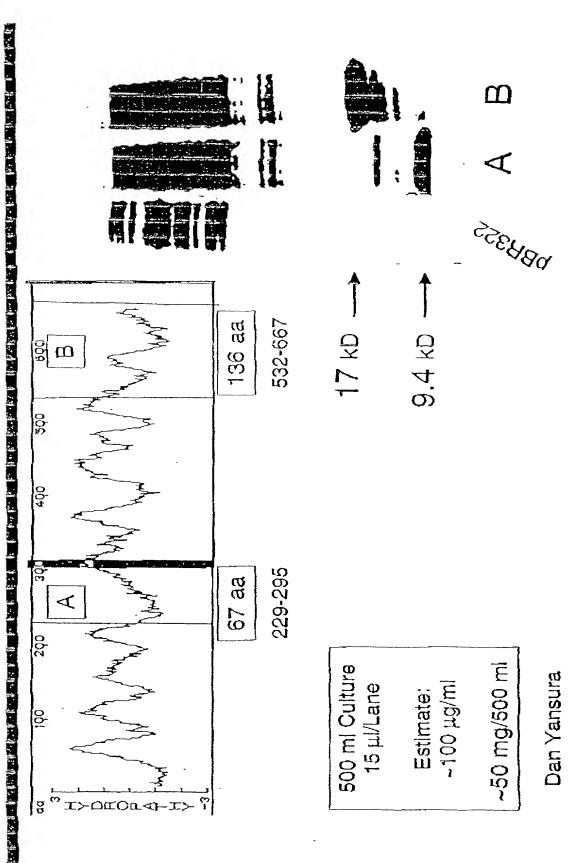
WJ TM #84 h.Stra6 Primer Set #4 1/4/00





Stra6 Peptide Expression in E. coli







HCT116 + HCT116 + HCT116 + HCT116 + Stra6 RNA Expression in Human Colon Carcinoma Cells +/- Retinoic Acid DMSO VD3 · vitamin D3 (1μM); ATRA · all-trans-retinoic acid (1 μM) HT-29 + 9cRA 9cRA · 9·cis-retinoic acid (1 µM) HT-29 + TM#75 (2/28/00) ATRA HT.29 + VD3 HT-29 + DMSO COLO205 COLO205 COLO205 COLO205 + 9cRA + VD3 + DMSO 4 7

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Relative Normalized Stra6 Units

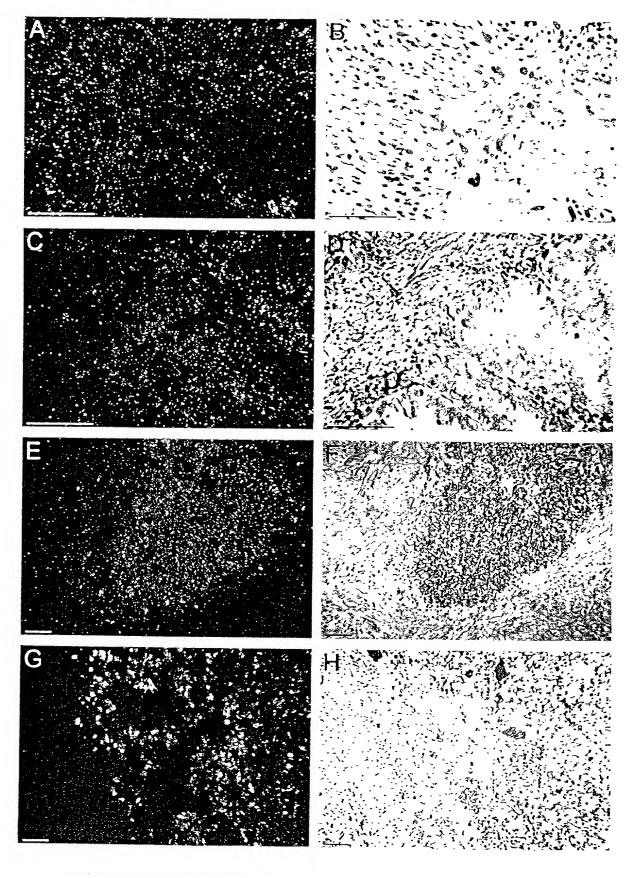
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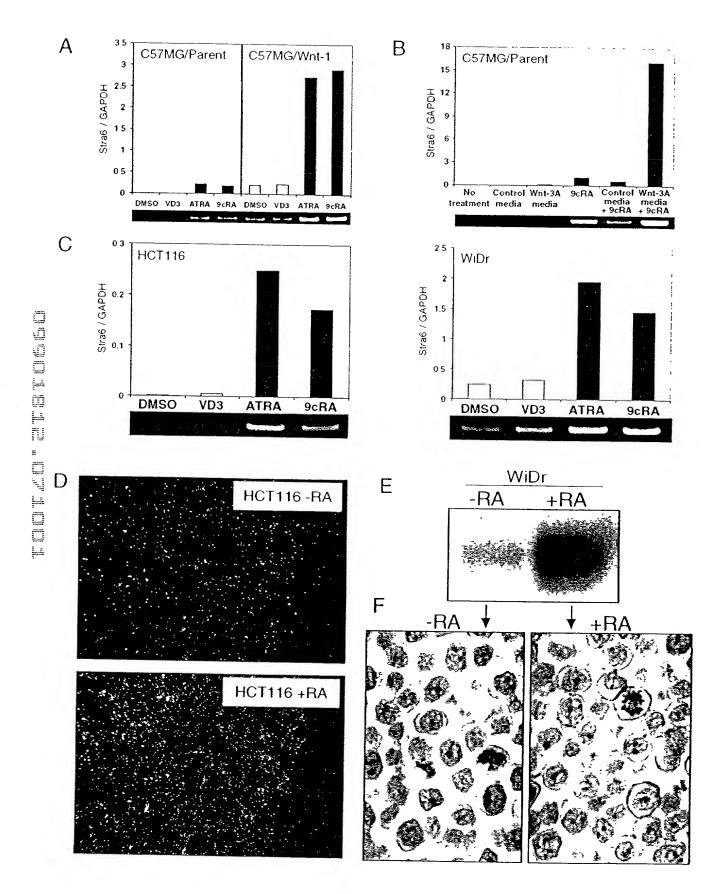
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Cell Line / Treatment

FIGURE 16





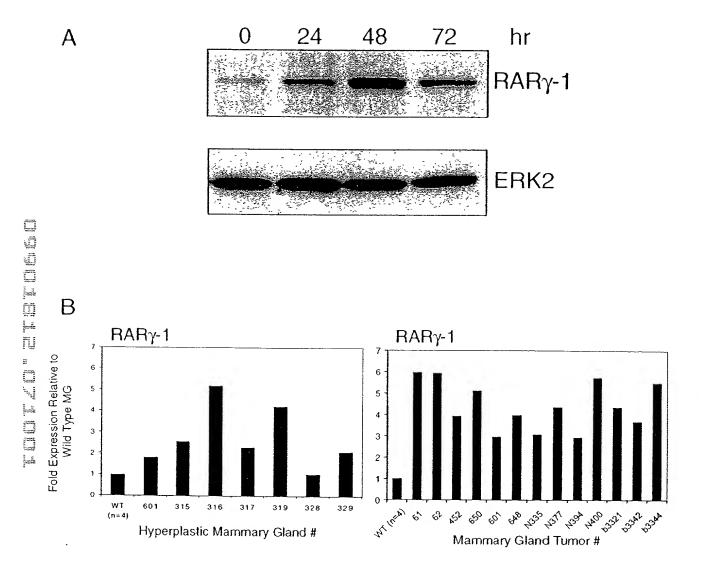
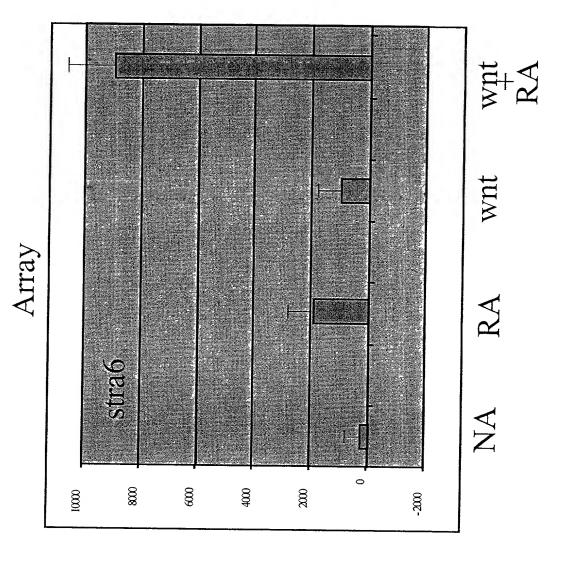
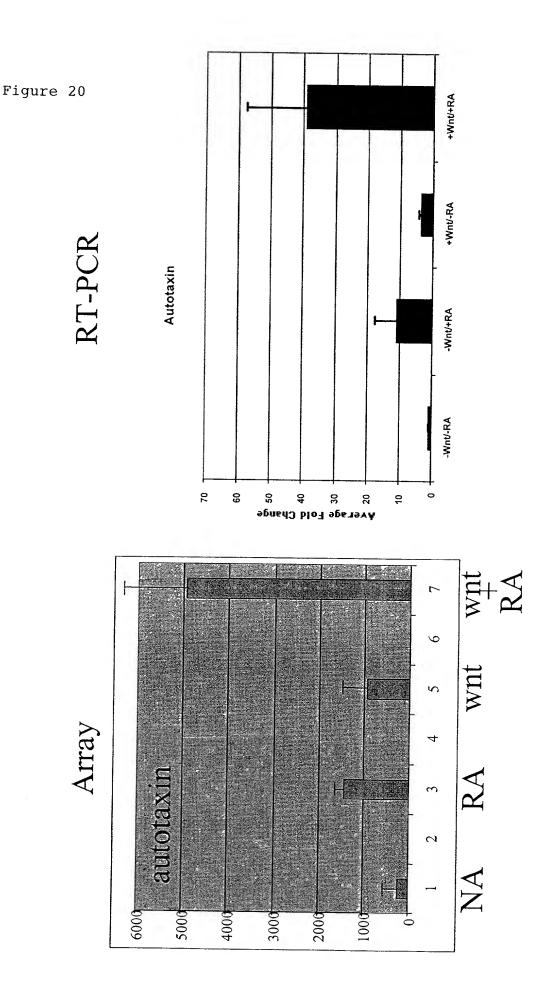
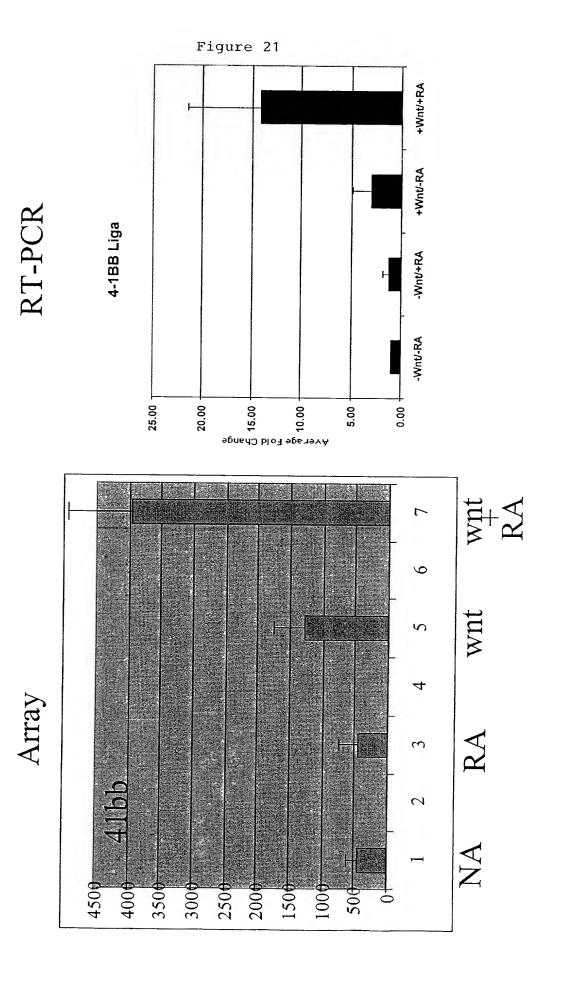
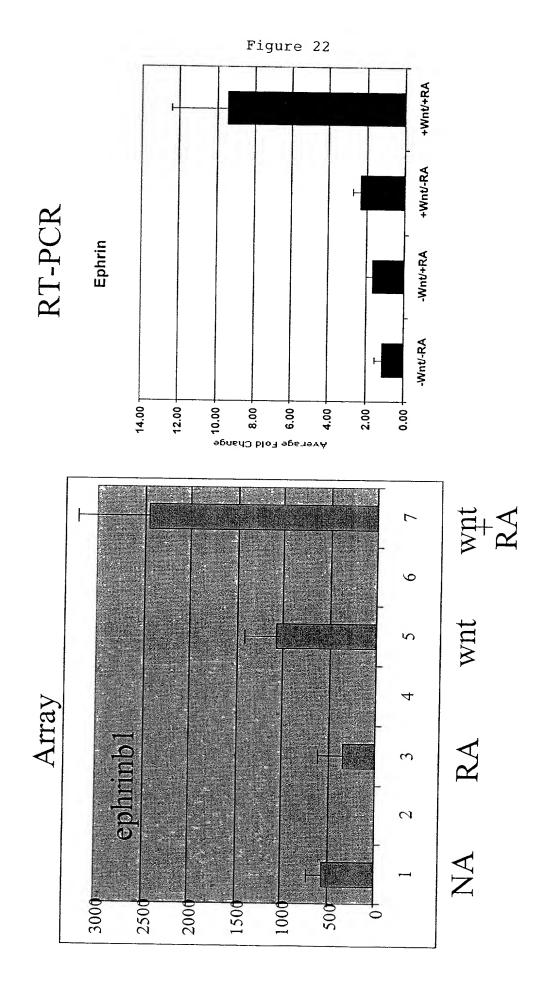


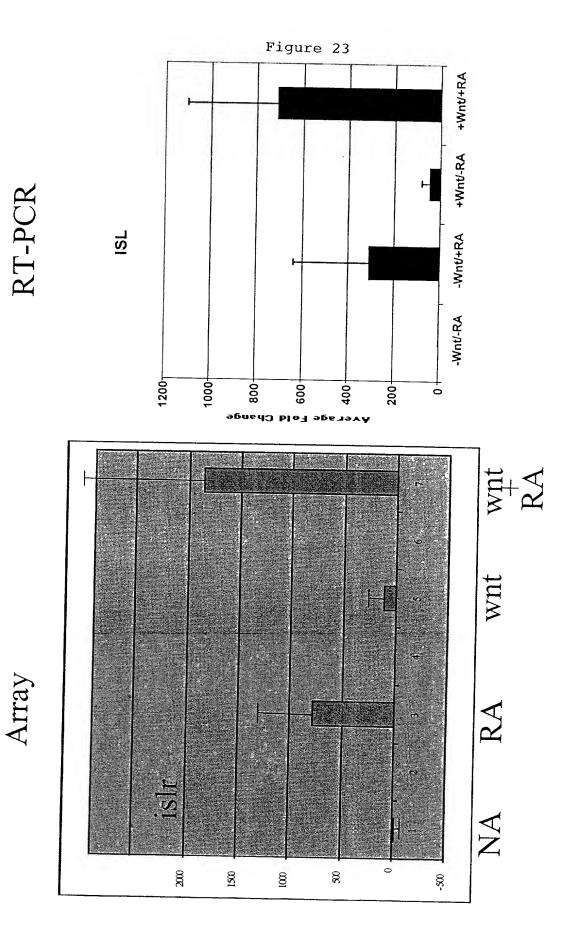
Figure 19











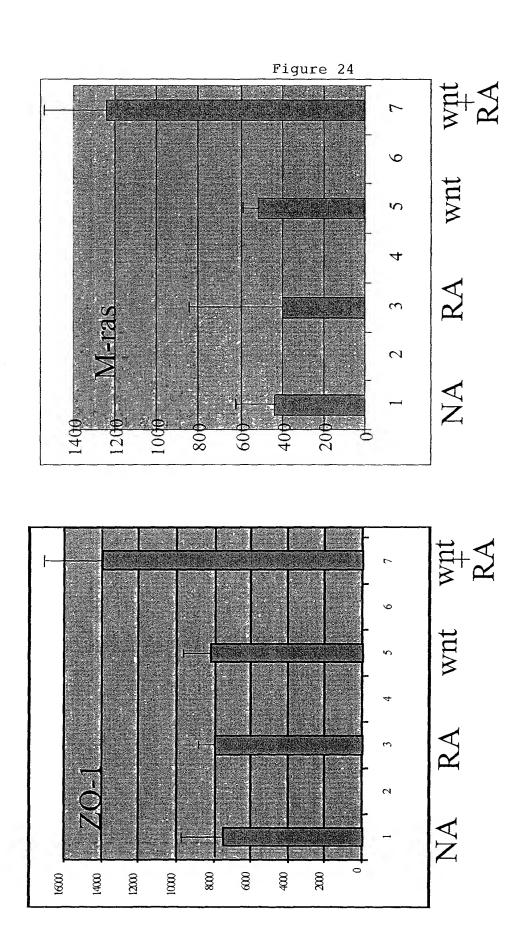
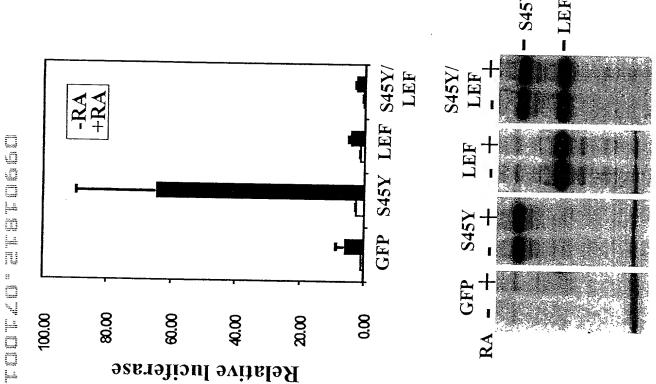


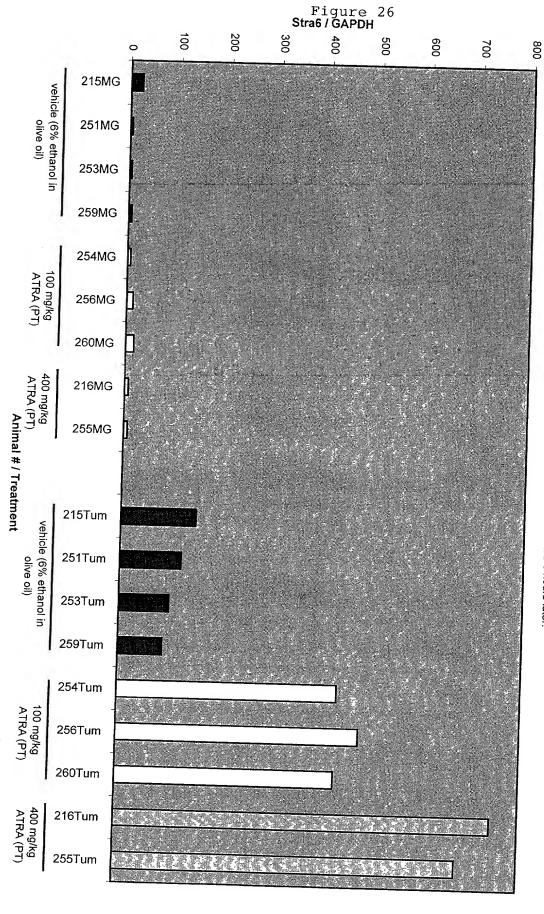
Figure 25 A

Figure 25 B



Stra6 mRNA in normal mouse mammary gland and Wnt-1mammary gland tumors

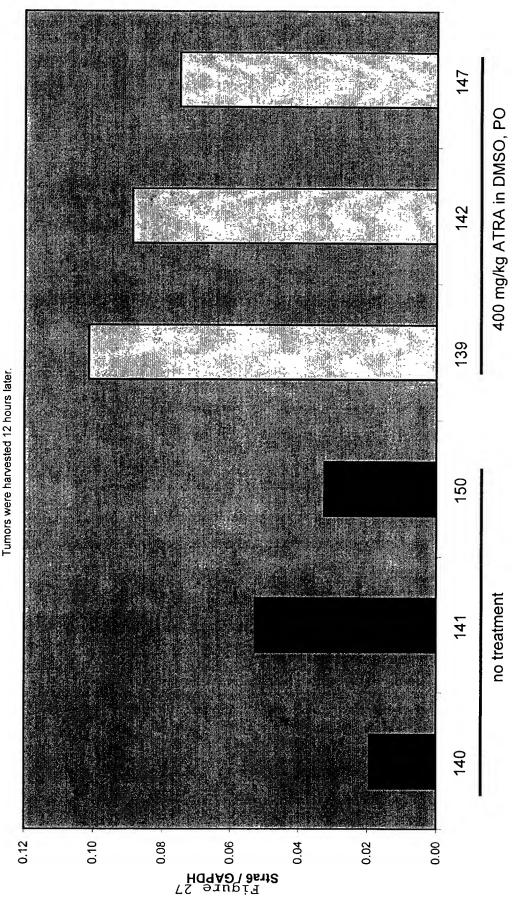
Nude mice bearing Wnt-1 tumor explants were given ATRA peri-tumorally (PT) at 100 and 400 mg/kg. Tumors and normal adjacent mammary glands were harvested 8 hours later.





Stra6 mRNA in WiDr xenografts from mice dosed with ATRA (400 mg/kg)

Nude mice bearing WiDr xenografts were given ATRA per orum (PO) at 400 mg/kg. Tumors were harvested 12 hours later.



Animal # / Treatment